## Biosciences

# Macromolecular Crystallography

### Automatic sample mounting and alignment system for macromolecular crystallography at the ALS
Snell, G., G. Meigs, C. Cork, T. Earnest

### Cocrystal structure determination of a pseudouridine synthase, a nucleotide-flipping RNA-modifying enzyme, at ALS Beamline 5.0.2
Hoang, C., A.R. Ferré-D'Amaré

### Crystal structure of nitric oxide synthase bound to nitroindazole reveals a novel inactivation mechanism
Raman, C.S., H. Li, P. Martásek, G. Southan, B.S.S. Masters, T.L. Poulos

### FTIR spectroscopy of bacteriorhodopsin microcrystals at Beamline 1.4
Betancourt, F., S. Rouhani, M. Facciotti, F. Burkard, R. Glaeser

### Structural studies of chromosomal building blocks
Chakravarthy, S., R. Edayathumangalam, U.M. Muthurajan, R.K. Suto, C.L. White, K. Luger

### Suite of three protein crystallography beamlines with single superconducting bend magnet as the source
MacDowell, A.A., D. Cambie, D. Plate, R. Duarte, N. Kelez, H.A. Padmore, M. Howells, R. Celestre, E. Domning, J. Holton, C. Cork, T. Earnest, J. Dickert, G. Meigs, J. Krupnick, T. Alber, S. Berger

### The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins
Kazantsev, A.V., A.A. Krivenko, D.J. Harrington, R.J. Carter, S.R. Holbrook, P.D. Adams, N.R. Pace

# Automatic sample mounting and alignment system for macromolecular crystallography at the ALS

G. Snell, G. Meigs, C. Cork and T. Earnest
Berkeley Center for Structural Biology, Physical Biosciences Division
Lawrence Berkeley National Laboratory, Berkeley, CA 94720

R. Nordmeyer, E. Cornell, J. Jaklevic, D. Yegian and J. Jin
Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

## ABSTRACT

In an effort to realize high-throughput, automated data collection for macromolecular crystallography, we have developed and installed an automatic cryogenic sample mounting and alignment system for protein crystals. This system has been installed on BCSB/ALS beamline 5.0.3 at the Advanced Light Source. The automounter was installed and began testing in March of 2001 and has been in user operation since September, 2001 with reliable performance. To further increase throughput, we have also developed a sample transport/storage system based on "puck-shaped" casettes which can hold 16 samples each. Centering of a crystal can be done by the user through the remote controlled xyz goniometer head or automatically by a centering algorithm. Our near term goal is to achieve fully automated screening (i.e. mounting, centering and exposing) of all samples in the dewar (currently 64) and to increase the capacity of the automounter dewar. Compatibility with systems available at other beamlines and facilities is a necessary design criteria. Software for the advanced control of these experiments is also under development.

This work was supported by the National Institutes of Health.

Principal investigator: Thomas N. Earnest, Physical Biosciences Division, Lawrence Berkeley National Laboratory. Telephone: 510-486-4603. Email: tnearnest@lbl.gov.

# COCRYSTAL STRUCTURE DETERMINATION OF A PSEUDOURIDINE SYNTHASE, A NUCLEOTIDE-FLIPPING RNA-MODIFYING ENZYME, AT ALS BEAMLINE 5.0.2

C. Hoang and A.R. Ferré-D'Amaré
Division of Basic Sciences, Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue North, Seattle, WA 98109-1024, USA

## SUMMARY

Pseudouridine (Ψ) synthases catalyze the isomerization of specific uridines in cellular RNAs to pseudouridines and may function as RNA chaperones. TruB is responsible for the Ψ residue present in the T loops of virtually all tRNAs in the cell. The close eukaryotic homolog Cbf5/dyskerin is the catalytic subunit of box H/ACA snoRNPs that catalyze modification of ribosomal RNA. The 1.85 Å resolution structure of TruB bound to an inhibitor RNA [1] shows that this enzyme recognizes the preformed three-dimensional structure of the T loop. It gains access to its substrate uridyl residue by flipping out the nucleotide, and thus disrupts tertiary, or long-range, interactions between the T and D loops of tRNA. This is the first structure determination of an RNA base-modifying enzyme bound to a substrate.

## BACKGROUND

Pseudouridine (Ψ) is the most abundant modified nucleotide in RNA. The isomerization of U into Ψ involves breakage of the glycosidic bond connecting the ribose to the pyrimidine base, rotation of the detached base, and reconnection through C5 (Fig.1). These enzymes require no cofactors or external sources of energy.

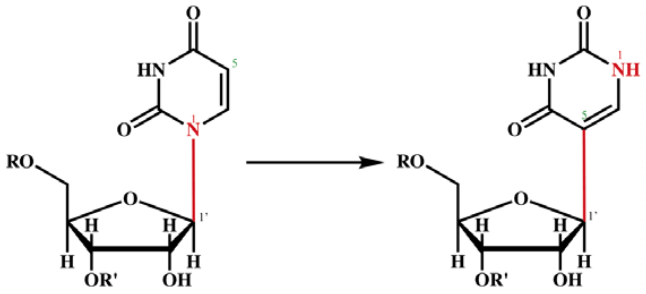


Figure 1. Isomerization of uridine (left) into Ψ catalyzed by Ψ synthases. Note how the glycosidic bond (in red) in uridine is replaced by a carbon/carbon bond in Ψ. R and R' denote the RNA chain to either side of the site of modification.

## EXPERIMENTAL DESIGN

In order to understand how a Ψ synthase recognizes its target RNA, and how the post-transcriptional catalytic transformation is achieved, we determined the structure of *Escherichia coli* TruB in complex with an RNA-based inhibitor (Fig. 2A). RNAs with a 5-fluorouracil (5FU) residue at the site of modification are tight mechanism-based inhibitors of tRNA Ψ synthases [2]. Since the regioselectivity and kinetics of pseudouridylation are the same on full-length tRNAs and RNAs comprised of the isolated T stem and loop (TSL RNAs), all determinants of specific Ψ55 synthase-tRNA recognition must lie within this segment of tRNAs [3]. A 22-nucleotide (nt) TSL with 5FU at the site of modification produced well-ordered cocrystals. The TruB cocrystal structure reveals that this Ψ synthase gains access to its substrate by flipping out nucleotide 55 of tRNA. In the TruB-TSL complex, the bases of nucleotides 55, 56, and 57 are everted from the position they would assume within the helical stack of isolated, folded tRNA (Fig. 2B). In folded tRNAs, these three nucleotides in the T loop contact the D loop to stabilize the tertiary structure of the RNA.

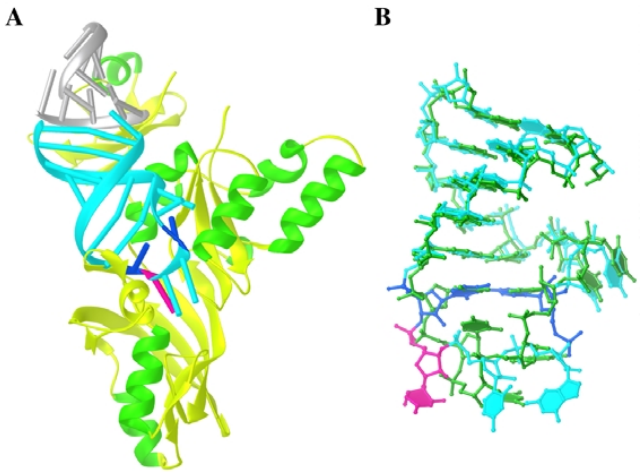


Figure 2. Three-dimensional structure of the Ψ synthase TruB complexed to a T stem-loop RNA. **A**.Ribbon representation of the complex. Protein helices are dark green, strands and loops are light green. The TSL RNA is light blue, except for nucleotides U54 and A58 that make a conserved reverse Hoogsteen pair colored dark blue, and U55 (the site of pseudouridylation) in purple. A segment of a symmetry-related RNA that extends the A-form helix is shown in light gray. **B.** Superposition of the TSL bound to TruB (colored as in Figure 1) with the corresponding residues from the structure of intact, folded $tRNA^{Phe}$ (in green). Three nucleotides at the apex of the T loop are flipped out of the helical stack by binding to TruB.

## STUDIES CONDUCTED AT THE ADVANCED LIGHT SOURCE

Our TruB cocrystals have the symmetry of space group *C*2, and unit cell dimensions $a = 145.05$ Å, $b = 40.36$ Å, $c = 77.99$ Å and $\beta = 110.60°$. Experimental phases were calculated at 2.0 Å resolution using MAD data measured at beamline 5.0.2 using a cocrystal containing a selenomethionine version of TruB. The MAD diffraction data were collected at three wavelengths corresponding to the peak, inflection point, and high energy remote for selenium as determined from a flurorescence energy scan of the actual crystal. Given the low symmetry of the crystal, in order to obtain complete anomalous data sets, 360° of data were collected at the both the peak and remote wavelengths. A 180° of data were collected at the inflection point. The three X-ray energies remained very stable over the eleven hours of data collection. 'Solvent flattening' and phase extension to 1.85 Å resolution produced an experimental electron density

map into which most of the protein and RNA residues could be built unambiguously (Fig. 3). The structure has been refined to an *R* factor of 18.4% and a free *R* factor of 21.2%.

The cocrystal structure of TruB reveals for the first time how a Ψ synthase recognizes its substrate and suggests how it may function in promoting RNA folding [1]. This high resolution structure is the starting point for unravelling the mechanism of action of these phylogenetically conserved enzymes, and also for exploring their possible roles in maturation and assembly of RNAs.

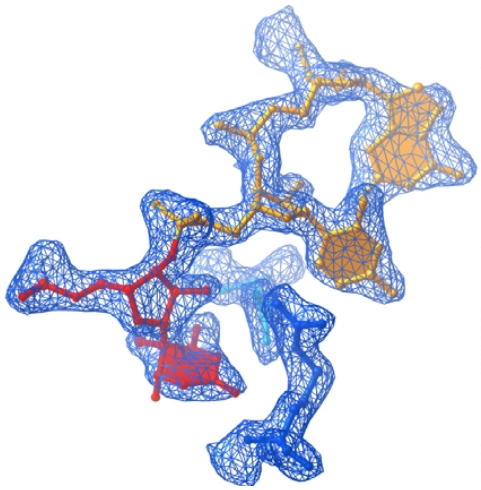

Figure 3. Portion of the 1.85 Å resolution 'solvent flattened' MAD experimental electron density map corresponding to part of the active site of TruB, contoured at 1.5 standard deviations above mean peak height.

## ACKNOWLEDGMENTS

We thank T. Earnest and K. Henderson for help at Advanced Light Source (ALS) beamline 5.0.2; J. Bolduc, P. Heath and B. Shen for crystallographic and computational support; and S. Biggins, D. Gottschling, H. Houbaivy, M. Roth, P. Rupert, S. Sigurdsson, J. Simon, B. Stoddard, G. Varani and D. Wilson for discussions.

## REFERENCES

1. C. Hoang and A.R. Ferré-D'Amaré, "Cocrystal structure of a tRNA Ψ55 pseudouridine synthase: nucleotide flipping by an RNA-modifying enzyme," Cell **107,** 929 (2001).
2. T. Samuelsson, "Interactions of transfer RNA pseudouridine synthases with RNAs substituted with fluorouracil," Nucleic Acids Res. **19**, 6139 (1991).
3. X. Gu, M. Yu, K. M. Ivanetich and D.V. Santi, "Molecular Recognition of tRNA by tRNA pseudouridine 55 synthase," Biochemisty **37,** 339 (1998).

This work was supported by grants from the National Institutes of Health and the Rita Allen Foundation to A.R.F., and from the American Cancer Society to the Fred Hutchinson Cancer Research Center (FHCRC). Access to ALS beamline 5.0.2 as part of the principal research consortium was made possible by general support from the FHCRC. A.R.F. is a Rita Allen Foundation scholar.

Principal Investigator: Adrian R. Ferré-D'Amaré, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109-1024. Email: aferre@fhcrc.org. Telephone: 206-667-3622.

# Crystal structure of nitric oxide synthase bound to nitroindazole reveals a novel inactivation mechanism

C. S. Raman,[*] Huiying Li,[*] Pavel Martásek,[†] Garry Southan,[¶]
Bettie Sue S. Masters,[†] and Thomas L. Poulos[*]

[*]Departments of Molecular Biology & Biochemistry and Physiology & Biophysics and the Program in Macromolecular Structure, University of California, Irvine, California 92697

[†]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229

[¶]Inotek Corp., Beverly, MA 01915.

Nitric oxide is generated under normal and pathophysiological conditions by three distinct isoforms of nitric oxide synthase (NOS). A small-molecule inhibitor of NOS (3-Br-7-nitroindazole, 7-NIBr) is profoundly neuroprotective in mouse models of stroke and Parkinson's disease. We report the crystal structure of the catalytic heme domain of endothelial NOS complexed with 7-NIBr at 1.65 Å resolution. We also present two crystal structures of eNOS complexed with either 7-nitroindazole-2-carboxamidiine or N-(4-chlorophenyl)-N'-hydroxyguanidine that reveal how alterations at the substrate site facilitate 7-NIBr and structurally dissimilar ligands to occupy the cofactor site. The x-ray diffraction data reported here were in part collected at Beamline 5.0.2 at ALS. Critical to the binding of 7-NIBr at the substrate site is the adoption by eNOS of an alternate conformation, in which a key substrate binding residue, Glu-363, swings out toward one of the heme propionate groups. Perturbation of the heme propionate ensues and eliminates the cofactor tetrahydrobiopterin-heme interaction. In fact, 7-NIBr selects for the catalytically incompetent Glu-363 rotamer and by binding to the substrate site locks this conformation in place. By competing simultaneously for both the substrate and cofactor binding sites, 7-NIBr is able to occupy one site and subsequently alter the specificity of a second site. Structural analyses of the 7-NIBr-bound eNOS structure teaches us that designing an inhibitor, which avoids H-bonded contact with one of the heme propionates and concurrently selects for the alternate Glu-363 rotamer, can serve as a potential template for designing drugs with isoform specificity. This is because such compounds dramatically weaken the affinity of the cofactor site for $H_4B$ and subsequently make it promiscuous. Therefore, one can take advantage of the small but significant differences at the substrate and cofactor binding sites toward designing bifunctional drugs with isoform selectivity.

This work was supported by NIH grants GM 57353 (TLP), and GM52419 (BSSM) and Robert A. Welch Grant No. AQ-1192 (BSSM).

Contact person: Dr. Huiying Li, Dept. MB&B UCI Tel: (949)-824-4322 Email: hli@bragg.bio.uci.edu

# FTIR spectroscopy of bacteriorhodopsin microcrystals at Beamline 1.4

F. Betancourt[1], S. Rouhani[2], Marc Facciotti[3], F. Burkard[1], and R. Glaeser[1,2,3]

[1]Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA

[2]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA

[3]Biophysics Group, University of California, Berkeley, California 94720, USA

Bacteriorhodopsin (bR) is the sole protein component of the purple membrane of *Halobacterium salinarium* [1]. The function of bR *in vivo* is to convert solar energy into a pH gradient across the cell membrane which the organism uses to drive ATP synthesis [2]. Bacteriorhodopsin undergoes a light-induced cycle of physicochemical changes for for every proton it pumps out of the cell. The photocycle of bR has been well-characterized by both visible and IR spectroscopy. The major intermediates are identified as the K, L, M, N and O intermediates, and each has a distinct visible color and a distinct IR spectrum [3]. The Schiff base that connects the side chain of Lys 216 to the retinal molecule buried within the core of the apoprotein [4] is deprotonated upon formation of the M intermediate, and reprotonated when the M intermediate decays. Since access to the Schiff base switches from the extracellular side of the membrane to the cytoplasmic side between these two proton transfer events, the M intermediate is of particular interest.

High resolution x-ray diffraction experiments on microcrystals of bR have recently become possible, through the discovery by Landau and Rosenbusch that the solubilized protein can be crystallized from the bicontinuous lipid-water gel that is formed by mono-olein [5]. Structural studies on intermediate states of the photocycle thus become a high priority, allowing the visualization of the structural changes that are responsible for converting light energy into a proton-motive force.

Previously we collected high resolution x-ray diffraction data from crystals of wild type bR trapped in both the L and M states by illumination at low temperature, using Fourier Transform IR (FTIR) spectroscopy to confirm the identity of the photointermediate[6]. This year we turned our attention this year to the F219L mutant of bR, which forms N, the intermediate following M, more readily than does the wild type protein[7]. We have succeeded in characterizing two photointermediates of the bacteriorhodopsin mutant F219L by means of FTIR spectroscopy. At 213K an early phase of the M photostate is trapped, whereas at 173K the IR signature strongly suggests that the L photostate is trapped. Further studies of this mutant are planned, with an eye towards trapping the N intermediate.

**ACKNOWLEDGMENTS**

We thank Dr. Michael Martin for his continued assistance at Beamline 1.4.3.

**REFERENCES**

1. Oesterhelt, D. & Stoeckenius, W. Rhodopsin-like protein from the purple membrane of Halobacterium halobium. *Nature New Biology* **233**, 149-152 (1971).
2. Racker, E. & Stoeckenius, W. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. *Journal of Biological Chemistry* **249**, 662-663 (1974).
3. Lozier, R. H., Bogomolni, R. A. & Stoeckenius, W. Bacteriorhodopsin: a light-driven proton pump in *Halobacterium halobium*. *Biophysical Journal* **15**, 955-962 (1975).
4. Henderson, R. et al. Model for the structure of bacteriorhodopsin based on high-resolution electron cyro-microscopy. *Journal of Molecular Biology* **213**, 899-929 (1990).
5. Landau, E. M. & Rosenbusch, J. P. Lipidic cubic phases: A novel concept for the crystallization of membrane proteins. *Proceedings of the National Academy of Sciences USA* **93**, 14532-14535 (1996).
6. Facciotti, M. T. et al. Structure of an early intermediate in the M-state phase of the bacteriorhodopsin photocycle. *Biophysical Journal* **81**, 3442-3455 (2001).
7. Vonck, J. A three-dimensional difference map of the N intermediate in the bacteriorhodopsin photocycle: Part of the F helix tilts in the M to N transition. *Biochemistry* **35**, 5870-5878 (1996).

This work was supported NIH grant GM51487.

Principal investigator: Felicia Betancourt, Advanced Light Source, Lawrence Berkeley National Laboratory. Email: fmh@xtalu.lbl.gov. Telephone: 510-486-6597.

# Structural studies of chromosomal building blocks

Srinivas Chakravarthy, Rajeswari Edayathumangalam, Uma M. Muthurajan, Robert K. Suto, Cindy L. White, Karolin Luger
Colorado State University (CSU), Department of Molecular Biology, Fort Collins, CO-80523-1870

## INTRODUCTION

The fate of a multi-cellular organism depends on the timed and coordinated readout of its genes. At the molecular level, this requires the accurate transcription of a subset of genes from the total complement that is present in each cell. Consequently, certain diseases and developmental disorders are associated with and even caused by aberrant gene expression. The DNA of a single mammalian cell is over two meters in length, but compacts in the cell nucleus to nearly one millionth of this dimension by a hierarchical scheme of folding and compaction into a highly dynamic protein-DNA assembly termed chromatin. Activation of a gene requires its identification within compacted chromatin. Local unpacking and remodeling of chromatin allows access of regulatory proteins and the transcription machinery, resulting in gene activation. Thus, the organization of DNA in chromatin has profound implications for the regulation of gene expression.

High-resolution crystal structures of nucleosome core particles (NCP) from *Xenopus laevis* reveal an octameric histone core around which 147 base pairs of DNA are wrapped in 1.65 superhelical turns [1] (Fig. 1). The histone octamer itself is composed of two copies each of the four histone proteins H2A, H2B, H3, and H4. Massive distortion of the DNA is brought about by the tight interaction between the rigid framework of the histone proteins with the DNA at fourteen independent DNA binding locations [1, 2]. We have previously determined the structure of a nucleosome containing an essential histone variant, from data collected at the ALS [3]. These studies are now being extended to study nucleosomes from other organisms, and are being expanded into new areas.

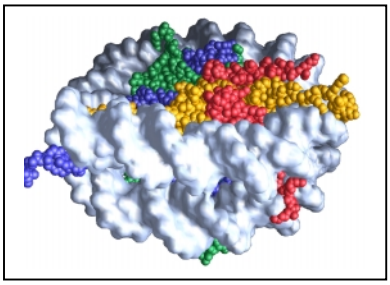

Fig. 1: Structure of the NCP. DNA is shown as a molecular surface in white, protein is shown as a space-filling model in red (H2B), yellow (H2A), blue (H3), and green (H4).

## STRUCTURE OF THE YEAST NUCLEOSOME CORE PARTICLE

Fundamental differences between the yeast genome and that of higher organisms suggest that chromatin might be organized in a different manner in yeast. Yeast is a unicellular organism whose entire genome is only ~ 0.5 % the size of that of humans, and its histone proteins are the most divergent among all eukaryotes. The crystal structure of the nucleosome core particle from *Saccharomyces cerevisiae* reveals that the structure and function of this fundamental complex is conserved between single-cell organisms and metazoans [4]. Our results show that yeast nucleosomes are likely to be subtly destabilized as compared to nucleosomes from higher eukaryotes, consistent with the notion that much of the yeast genome remains constitutively open during much of its life cycle. Importantly, minor sequence variations lead to dramatic changes in the way in which nucleosomes pack against each other within the crystal lattice. This has important implications for our understanding of the formation of higher-order chromatin structure and its modulation by post-translational modifications. Finally, the yeast nucleosome core particle provides a structural context by which to interpret genetic data obtained from yeast. Coordinates have been deposited with the Protein Data Bank under accession number 1ID3.

## SITE-SPECIFIC RECOGNITION OF NUCLEOSOMAL DNA

The ability of a sequence-specific DNA binding protein to recognize its cognate site in chromatin is restricted by the structure and dynamics of nucleosomal DNA, and by the translational and rotational position of the histone octamer with respect to the binding site. Here we use high-affinity, sequence-specific pyrrole-imidazole polyamides as molecular probes for DNA accessibility in nucleosomes. Sites on nucleosomal DNA facing away from the histone octamer, or even partially facing the histone octamer, are fully accessible and the nucleosomes remain fully folded upon binding. Polyamides only fail to bind where sites are completely blocked by interactions with the histone octamer.

We have determined several high-resolution crystal structures of nucleosome core particles in complex with different hairpin pyrrole-imidazole polyamides (Fig. 2). These structures represent the first nucleosome – ligand co-crystal structures, and provide the first insight into the molecular details of base-specific DNA recognition of nucleosomal DNA. We showed that the binding of ligand does not disrupt any interactions between histones and DNA [5]. All polyamides fit snugly in the minor groove of nucleosomal DNA. Extensive hydrogen bonding between ligand and bases (in addition to non-specific hydrophobic interactions) accounts for the observed high specificity of binding, according to the specificity rules stated by Dervan and colleagues [6]. Local structural changes are imparted on the nucleosomal DNA upon binding. The ligand-induced changes in DNA topology are compensated for by conformational changes in the DNA that are distant from the binding site.

The observed effects of complex formation on the structure of polyamides and nucleosomal DNA have implications for the binding of sequence-specific transcription factors to nucleosomal DNA, and demonstrate a surprising flexibility and plasticity of nucleosomal DNA. Our results demonstrate that much of the DNA in the nucleosome is freely accessible for molecular recognition.

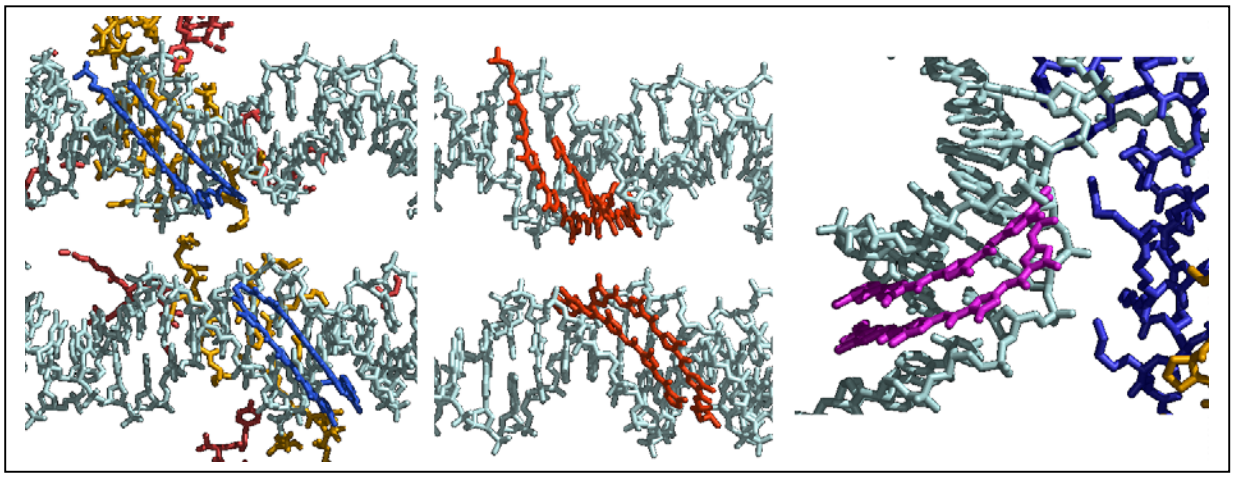

**Fig. 2: Close-up view of three NCP-polyamide co-crystal structures.** View is from the outside of the nucleosome. Polyamides with different sequence specificities are shown in blue, orange, and magenta, respectively.

This work was funded by grants from the NIH (GM61909), March of Dimes (99-853), Human Frontiers Programs Organization (00059), and by the Searle Scholar award to KL.

Principal Investigator: Karolin Luger, Colorado State University, 970-491-6405, kluger@lamar.colostate.edu.

**REFERENCES:**


*1* Luger, K. *et al*. (1997) *Nature* 389, 251-259

*2* Luger, K. and Richmond, T. J. (1998) *Current Opinion in Structural Biology* 8, 33-40

*3* Suto, R. K., Clarkson, M. J., Tremethick, D. J. and Luger, K. (2000) *Nat Struct Biol* 7, 1121-1124

*4* White, C. L., Suto, R. K. and Luger, K. (2001) *Embo J* 20, 5207-18.

*5* Gottesfeld, J. M. *et al*. (2001) *J Mol Biol* 309, 625-39.

*6* Dervan, P. B. (2001) *Bioorg Med Chem* 9, 2215-35.

# Suite of Three Protein Crystallography Beamlines with Single Superconducting Bend Magnet as the source.

A.A.MacDowell[1], D.Cambie[2], D.Plate[2], R.Duarte[2], N.Kelez[2], H.A.Padmore[1], M.Howells[1], R.Celestre[1], E.Domning[1], J.Holton[3], C.Cork[3], T.Earnest[3], J.Dickert[3], G.Meigs[3], J.Krupnick[1], T.Alber[4], S.Berger[4].

[1]Advanced Light Source Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA
[2]Engineering Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA
[3]Physical Biosciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720, USA
[4] Dept. of Molecular and Cell Biology, 229 Stanley Hall, University of California, Berkeley, California, 94720, USA

## INTRODUCTION

At synchrotrons around the world Protein Crystallography beamlines are being built at a rapid pace as it has been realized that this technique can rapidly elucidate protein structures, which can provide one of the foundation blocks for the understanding of life. Many Protein Crystallography beamlines are required as there are many tens of thousands of protein structures to solve. Here at the Advanced Light Source (ALS), a consortium from the Universities of Berkeley and San Francisco have constructed beamline 8.3.1, quickly followed by the Howard Hughes Medical Institute funding the construction of two more identical beamlines (8.2.1 and 8.2.2). All three beamlines are adjacent and use as a source one of the three newly installed 5 Tesla single pole Superconducting-bending magnets. Beamline 8.3.1 is in operation and the other 2 beamlines are finalizing the commissioning process. This document will briefly describe these beamlines and their initial performance.

## BEAMLINE DESIGN

Initial work was carried out in 1998 by the University of Berkeley (UCB) group on the test beamline 7.3.3 to determine the feasibility of carrying out Protein Crystallography on regular ALS bending magnets. Complete multiple anomalous dispersive data sets were collected to 1.9Å resolution in ~ 10hours on the non- optimized test beamline [1]. By optimizing the beamline and switching from a regular warm magnet dipole source to a 5 Tesla Superconducting dipole source [2], it would be expected that scan times would drop to ~ 1hour. Speed is increasingly becoming very important for the Protein Crystallography process both due to the large number of protein structures to be solved and the requirement to scan for good samples amongst the many poor ones during the iteration of crystallization procedures.

The simplistic basis for the beamline design was to put as many 6-16 KeV photons through a 100μm diameter pinhole as possible consistent with reasonable cost and robust performance. The optimized beamline design schematic is shown in figure 1. 1.5mrad (h) x 0.5mrad (v) of radiation is accepted by the nickel-plated water-cooled invar mirror (M1), 6.5m from the source. It is shaped into a parabolic cylinder by means of a bending mechanism. Parallel light in the vertical plane is directed into a water-cooled double crystal monochromator (Kohzu

Co.) 18m from the source. The exiting monochromatic light is focused by a toroidal mirror (M2) (21.53m) onto the sample in the hutch with a horizontal demagnification of 2. The end stations are of the mini-hutch type allowing for changing samples by reaching through a sliding door. Ray tracing indicated that using a dipole source size of 230μm (h) x 23μm (v) fwhm resulted in a focused spot size of 150μm (h) x 67μm (v) fwhm with a divergence of 3mrad (h) x 0.33mrad (v).

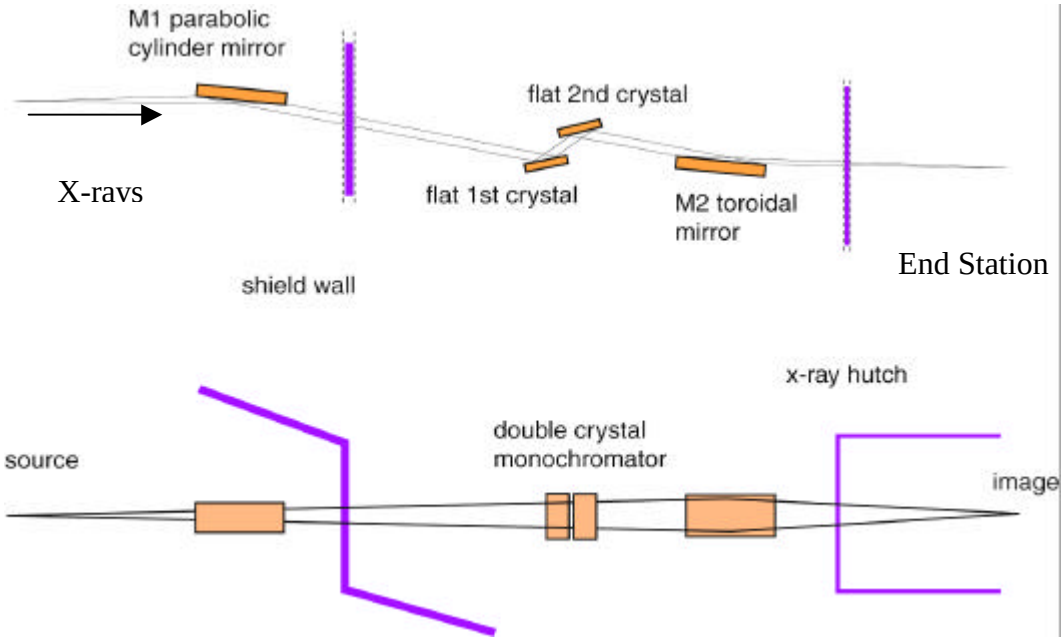


**Figure 1.** Schematic layout of the new Protein Crystallography Beamlines with a Superbend dipole magnet source. Mirror grazing angles are 4.5mrads.

## BEAMLINE PERFORMANCE

The focus spot size at the sample has been measured on the 3 beamlines as ranging from 150μm (h) x 120-160μm (v). This is slightly higher in the vertical than expected and is most likely due to figure and roughness problems that arose during the manufacture of the M1 mirrors. New ones are currently being fabricated. The flux through the 100um pinhole is calculated as 2 x $10^{11}$ hυ/sec at 12.4KeV (ALS =400mA), but the actual flux measured is less being in the range of 4-8 x $10^{10}$ hυ/sec at) for the 3 beamlines. This too is expected to improve when the new M1 mirrors are installed. The x-ray convergence angles onto the sample were 1.5mrad (h) x 0.33mrad (v). This is still a very respectable flux and very comparable to the 5.0.2 Protein Crystallography beamline, which uses a 38-pole 2.1 Tesla wiggler magnet insertion device. This is a high power, low brightness source and yields a sample flux of 7x$10^{10}$ hυ/sec at 12.4KeV for the same conditions as used on the Superbend beamlines. This confirms the original concept that the high brightness low power Superbend dipole source can compete with high power low brightness insertion devices for selected applications.
Beamline 8.3.1 has been operational for 2-3 months and has solved 17 structures. Beamlines 8.2.1 and 8.2.2 are undergoing the final commissioning.

## REFERENCES


1. T. Alber, J.M. Holton, J.Plecs, R. Rose, A.A.MacDowell, R.S. Celestre, H.A. Padmore, The practicality of an ALS bend magnet as the x-ray source for solving structural biology problems. Advanced Light Source Activity Report, Abstracts 1998
2. Advanced Light Source Activity Report 2001, Facilities Report Section.



This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, and Materials Science Division, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.



Principal investigator: Alastair MacDowell, Advanced Light Source, Ernest Orlando Lawrence Berkeley National Laboratory. Email: aamacdowell@lbl.gov. Telephone: 510-486-4276.

# The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins.

*Alexei V. Kazantsev*[*], *Angelika A. Krivenko*[*], *Daniel J. Harrington*[†], *Richard J. Carter*[‡], *Stephen R. Holbrook*[‡], *Paul D. Adams*[‡] *and Norman R. Pace*[*].

[*]*Department of MCD Biology, University of Colorado, Boulder, CO 80302*

[†]*Stanford Synchrotron Radiation Laboratory, Stanford University, Menlo Park, CA 94025*

[‡]*Physical Biosciences Division, Lawrence Berkeley National Lab, Berkeley, CA 94720*

RNase P, the ubiquitous endonuclease that catalyzes maturation of the 5'-end of tRNA, in Bacteria is a ribonucleoprotein particle composed of a large, catalytically active RNA and a small protein. Two major structural classes of RNase P RNA have been identified by phylogenetic comparative analysis, the A (ancestral) and B (Bacillus) types[1]. We have solved the X-ray crystal structure of the RNase P protein from the hyperthermophilic bacterium *T.maritima* at 1.3Å resolution[2]. This protein binds the A-type RNase P RNA.

In the crystal, the protein forms asymmetric dimers. In the light of substantial differences in the A and B-type RNAs, this protein structure bears remarkable similarity to the recently determined structures of RNase P proteins from organisms with B-type RNAs[3]. Structural conservation of potential RNA-binding elements in RNase P protein indicates that it binds to RNase P RNA primarily via direct contacts with the phylogenetically conserved core that is shared by A and B-classes of RNase P RNA.

In order to assess a potential physiological role for the protein dimerization, attempts to detect the dimerization in solution by light-scattering technique are currently in progress.

## REFERENCES

1. Frank D.R. and Pace N.R, 1998. Ribonuclease P: unity and diversity in a tRNA processing ribozyme. *Annu Rev Biochem* **67**:153-180.
2. Kazantsev, A.V. et al., The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins (in preparation).
3. Stams T. et al., 1998. Ribonuclease P protein structure: evolutionary origins in the translational apparatus. *Science* **280**:752-755.

This work has been supported by NIH grant GM34527.

Principal investigator: Norman R. Pace, Department of MCD Biology, University of Colorado, Boulder, CO 80302. Email: nrpace@colorado.edu. Telephone: 303-735-1808.